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Microbiologic and Clinical Evidence Supporting the Role of *Aeromonas caviae* as a Pediatric Enteric Pathogen

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Aeromonas caviae **was recovered as the sole potential enteric pathogen from the stools of 14 of 17 symptomatic children (10 younger than 1 year of age) while *Aeromonas hydrophila*, *Aeromonas sobria*, and *Plesiomonas shigelloides* were isolated once each. The infants from whom *A. caviae* was isolated all presented with a watery diarrhea lasting 1 to 3 weeks. None of these infants was breast-fed, and all had a stool pH of >7.5. All of the *A. caviae* isolates, including a reference strain (ATCC 15468), adhered to HEp-2 cells, and preliminary data showed that they produced a cytotoxin as well. Because *A. caviae* can survive at an elevated pH, as found in the gastrointestinal tract of formula-fed infants, and because of the adherence and cytotoxin production capabilities of the species, it should be regarded as an enteric pathogen in pediatric patients and most probably among adults as well.**

Mesophilic members of the genus *Aeromonas* have been associated with a variety of human infections. While the etiologic significance of *Aeromonas hydrophila*, *Aeromonas sobria*, and more recently *Aeromonas veronii* (12) in gastrointestinal tract infections has been supported, the role of *Aeromonas caviae* in this setting is disputed (3, 9, 13, 21). In our laboratory and in laboratories in diverse geographic locales (1, 6, 19), however, *A. caviae* is the most common aeromonad isolated from diarrheal stool specimens, especially from children (1, 8, 19). Despite this occurrence, the role of *A. caviae* as a gastrointestinal tract pathogen in pediatric patients is still unresolved.

During a 5-month period (May to October 1989), 16 *Aeromonas* isolates and 1 *Plesiomonas shigelloides* isolate were obtained from 17 children, mostly infants with a diarrheal illness. Fourteen of these isolates were *A. caviae*. Microbiologic study of these latter isolates and review of patient charts and clinical courses strongly suggest a role for *A. caviae* as a pediatric enteric pathogen.

MATERIALS AND METHODS

Eleven hundred stool specimens collected from pediatric patients (1 week to >10 years old) were processed for the isolation of *Aeromonas* and/or *Plesiomonas* species and other enteric pathogens including *Campylobacter* species by inoculating MacConkey, Hektoen-Enteric, and 5% sheep blood agars and a Campylobacter plate (BBL Microbiology Systems, Cockeysville, Md.). Routine cultures were incubated at 35°C for 24 h, while the Campylobacter plate was incubated in an atmosphere of increased CO_2 (CampyPak Plus; BBL Microbiology Systems). The cultures were screened for enteric pathogens and for *Aeromonas* species specifically by flooding the sheep blood agar plates with Kovacs oxidase reagent. Colonies rendering an evolving lavender color were immediately subcultured to blood agar and subjected to species identification through the use of MicroScan Gram-negative Combo (Travenol Laboratories, Inc., Mahwah, N.J.) augmented by the criteria of Janda (14). Additionally, each isolate was tested for its suicidal tendency in nutrient broth supplemented with 0.5% glucose (17) and for susceptibility to the vibriostatic agent 0/129. All the tests were performed at 30°C.

Stool pH. The pHs of stool specimens were determined with Hydrion pH paper (Micro Essential Laboratory, Brooklyn, N.Y.).

Adhesion assay. Log-phase cells of *Aeromonas* species harvested from nutrient broth were diluted in phosphate-buffered saline containing 0.5% minimal salt solution (PBSS) to a concentration of 2×10^6 CFU/ml as determined by plate count. Semiconfluent HEp-2 cell monolayers, grown for 36 h on glass cover slips in 12-well trays, were washed with 2 ml of PBSS, after which 1 ml of the *A. caviae* suspension was added. The trays were incubated at 37°C in 5% CO_2 for 90 min. Nonadhering bacteria were removed from the wells by washing four times with 2 ml of PBSS. The monolayer cells were then fixed in a methanol-acetic acid (3:1) mixture for 5 min. The cover slips were then mounted on a glass slide and Giemsa stained. Adhesion was assessed microscopically, and the number of adherent bacteria was determined by averaging the number of bacteria adherent to 25 HEp-2 cells.

Patient charts. The charts of all patients from whom an oxidase-positive species was recovered were reviewed for patient age, presenting symptoms and their duration, nature of diet, antibiotic administration, and management of the diarrheal illness.

RESULTS

The salient characteristics of each of the oxidase-positive species are shown in Table 1. All *A. caviae* isolates and the *A. sobria* isolates were suicidal, whereas *A. hydrophila* and *P. shigelloides* remained viable after overnight growth in the glucose-containing medium.

The 16 *Aeromonas* strains and the *P. shigelloides* strain were all isolated on primary culture (without enrichment or use of a selective medium) from the stools of symptomatic children ranging from 5 weeks to 10 years of age. Ten (62.5%) of the infants were less than 1 year of age; four (25%) were 1 to 2 years of age, and the last two (12.5%) were 2 and 10 years old. No other enteric pathogens were isolated with the oxidase-positive species.

Each of the tested *Aeromonas* species was capable of

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TABLE 1. Phenotypic characteristics of *Aeromonas* and *P. shigelloides* isolates

Characteristic	Result for[a]:			
	A. caviae (*n* = 14)	*A. hydrophila* (*n* = 1)	*A. sobria* (*n* = 1)	*P. shigelloides* (*n* = 1)
Suicide phenomenon	+	−	+	−
Oxidase production	+	+	+	+
Indole production	+	+	+	+
0/129 Vibriostatic agent	R	R	R	S
Hemolysis (5% sheep blood agar)	−	+	+	−
Glucose fermentation	+	+	+	+
Aerogenicity	−	+	+	−
Acetoin production	−	+	+	−
Esculin hydrolysis	+	+	−	−
Arabinose utilization	+	+	−	−
Salicin fermentation	+	+	−	−

[a] R, Resistant; S, susceptible.

TABLE 2. Adherence of *Aeromonas* species to HEp-2 cells

Aeromonas sp.	No. of strains:			
	Tested	With no. of adherent bacteria/HEp-2 cell		
		<10	10–20	>20
A. caviae	14	6	2	6
A. hydrophila	3	0	1	2
A. sobria	1	1	0	0

adhering to HEp-2 cells (Table 2). *A. hydrophila* appeared to adhere best, as assessed by the number of bacterial cells attached to individual monolayer cells. With *A. caviae*, all 14 strains tested showed adhering capability, although a gradation in adherence could be discerned, ranging from strains showing sparse adhering affinity (<10 bacteria per cell) to those showing a mantle of adherent bacteria encircling each HEp-2 cell (Fig. 1).

A. caviae was recovered from 14 of the 17 children, while *A. hydrophila*, *A. sobria*, and *P. shigelloides* were each recovered once (Table 3). Ten of the infants from whom *A. caviae* was isolated presented with gastrointestinal symptoms often consisting of acute gastroenteritis lasting 1 to 3 weeks. A clinical presentation was not indicated for the remaining four patients with *A. caviae*, while vague symptoms (e.g., loss of appetite) were recorded for the patients whose stool cultures grew the other three oxidase-positive species.

None of the infants was breast-fed; all were being maintained on bottle and or formula milk. Two of the infants with *A. caviae* were born prematurely (28 weeks of gestation), one infant presented with pyloric stenosis, and another was positive for antibody to human immunodeficiency virus. Three received antibiotics; two received trimethoprim-sulfamethoxazole, and one received ampicillin. The latter infant had diarrhea for 3 weeks despite ampicillin administration; in vitro studies showed the isolate to be resistant to ampicillin. The remainder of the patients received supportive care only, mainly through banana, rice, apple, and toast dietary supplementation and rehydration.

The submitted stool specimens were uniformly alkaline, with a pH of >7.5. The diarrheal stools were usually watery

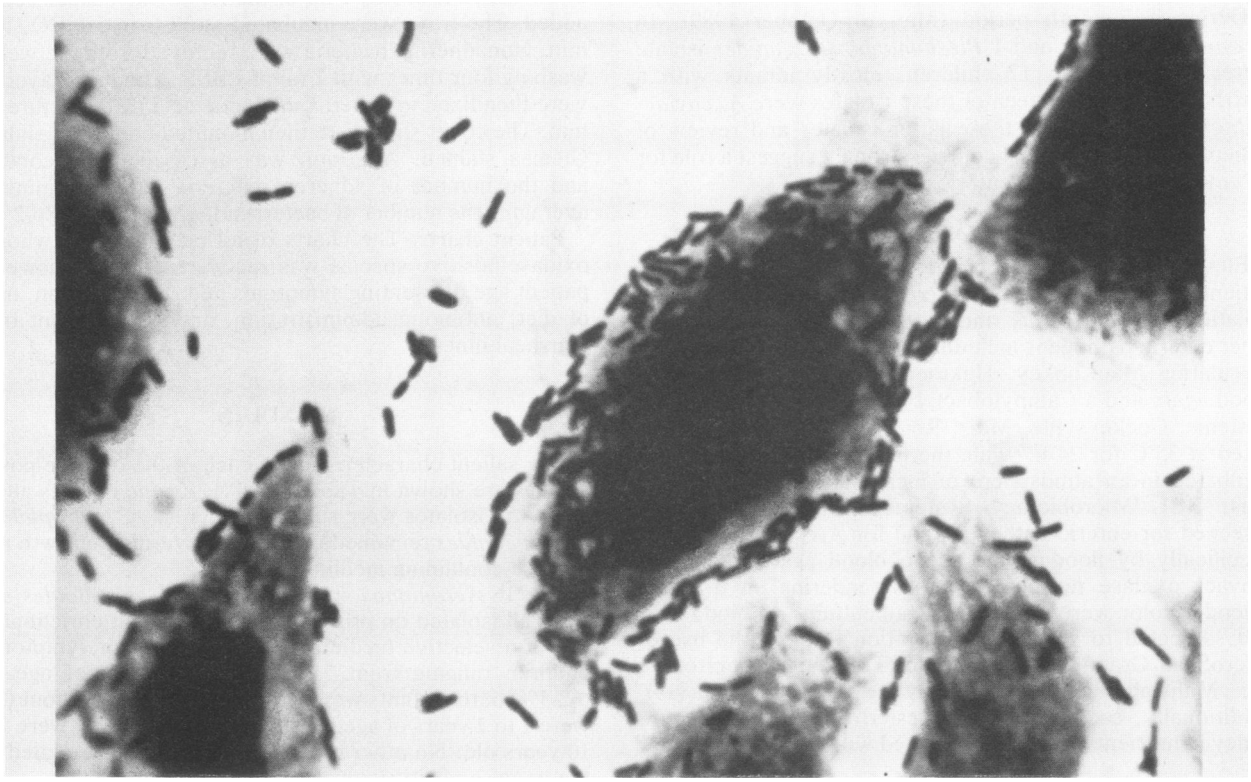

FIG. 1. Adherence of *A. caviae* to HEp-2 cells without internalization, as evidenced by the mantle of bacteria encircling each cell.

TABLE 3. Clinical correlation of *A. caviae* and other oxidase-positive species from feces of infants with diarrhea

Species isolated	No. of strains (%)	Age (no. of patients)	Clinical features[a]
A. caviae	14 (82)	5 wk–24 mo (12) 36 mo (1) 10 yr (1)	Watery diarrhea, two–six times daily; occasional blood lasting 3–14 days; two patients treated with TMP-SMX
A. hydrophila	1 (6)	21 mo	Watery, loose stool for 3–4 days; HIV^+
A. sobria	1 (6)	9 mo	Soft stool three times daily for 3 wk
P. shigelloides	1 (6)	24 mo	Watery, bloody diarrhea; 10–15 BM daily for 3 days; fever, 104°F (40°C); treated with TMP-SMX

[a] TMP-SMX, Trimethoprim-sulfamethoxazole; HIV, human immunodeficiency virus; BM, bowel movement.

and were being passed from three to seven times daily (two infants had 10 to 15 bowel movements daily) over a course of 3 to 21 days. Five stool specimens were positive for occult blood, three of which were from patients with *A. caviae*.

DISCUSSION

There are several criteria that must be met in order for *A. caviae* to be regarded as a gastrointestinal tract pathogen in pediatric patients.

Initially, *A. caviae*, subsequent to ingestion, must be able to colonize the gastrointestinal tract. We, as well as Carrello et al. (7), have shown that this bacterial species, analogous to other *Aeromonas* species, can adhere to HEp-2 cells in vitro. While the degree of adherence to HEp-2 cells varied among our *A. caviae* strains, all 14 strains recovered from the feces of symptomatic children adhered in our assay system. While absolute clinical relevance can not be ascribed to in vitro HEp-2 cell adherence, a correlation between enteropathogenicity and in vitro adherence does exist for numerous other bacterial species (6, 15).

Subsequent to, or concomitant with, colonization, a major factor bearing on *A. caviae* enteropathogenicity is the ability of the species to survive in the gastrointestinal tract. As shown previously (16, 18), *A. caviae* uniformly self-destructs (suicide phenomenon) in the presence of low pH induced under the action of acetic acid accumulation. In the present study, however, two factors conspire to ensure survival of *A. caviae* in the infant gastrointestinal tract: formula feeding and a resultant elevated stool pH of >7.5.

The microbial flora of the gastrointestinal tract of breast-fed infants comprises mainly *Bifidobacterium* species, which render the infant intestinal tract acidic (pH 5.1) by production of acetic and lactic acid by lactose fermentation (22). By way of contrast, the intestinal bacterial floras of the formula-fed infant are predominantly *Bacteroides* species, members of the family *Enterobacteriaceae*, and *Enterococcus* species and hence have higher pHs (pH 6.5) (4). While we did not investigate the feeding patterns of other symptomatic infants from whom an *Aeromonas* species was not isolated, all of our patients (infants) from whom *A. caviae* was isolated were formula fed and had a stool pH slightly above neutral. Interestingly, as both *A. hydrophila* (suicidal at 37°C) and *A. sobria* are also susceptible to acid-induced killing, elevated stool pH as a consequence of formula feeding also favors their survival in the infant gastrointestinal tract.

Correlative to the above, and additional testimony supporting the predominant recovery of *A. caviae* (in contrast to other mesophilic aeromonads) from stool specimens, is the adaptation of *A. caviae* for growth under anaerobiosis in low-nutrient alkaline environments (18). This biologic attribute of *A. caviae* may further ensure survival in the intestinal microenvironment of the formula-fed infant, wherein a lowered redox potential may prevail, as evidenced by the survival of *Bacteroides* species and other anaerobes (11).

In our series of experiments, as well as those of other investigators, *A. caviae* has been recovered from the stools of older children with symptoms of gastrointestinal disease, as well as from infants. Careful scrutiny of the data, when available, usually reveals that these children have an altered stool flora because of an underlying disease, severe malnutrition, or antibiotic administration, which apparently favors survival of *A. caviae*.

Further evidence supporting the role of *A. caviae* (as well as other mesophilic aeromonads) as a gastrointestinal tract pathogen in pediatric patients may be derived from our findings, which include the isolation of *A. caviae* as the sole enteric pathogen from infants with gastroenteric symptoms. While viruses were not specifically sought, none of the infants presented with vomiting and fever, which suggest a viral etiology in this age group (21). Further, several infants had bloody stool, which also argues against a viral etiology (10). Additionally, when infants were treated with anti-infectives to which *A. caviae* was susceptible, e.g., trimethoprim-sulfamethoxazole, resolution of symptoms promptly occurred. Although we did not determine the carriage rate of *A. caviae* in control subjects with no symptoms, carriage rates for mesophilic *Aeromonas* species in children ranged from 0.5 to 2.1% (2, 8, 19). In each instance, as in the present study, the isolation rate for *A. caviae* was greatest for children 0 to 5 years of age. Unlike our analysis, however, no information was given as to the feeding patterns of the children studied.

The remaining question to be resolved is the pathogenesis of *A. caviae*-induced gastroenteritis. Two plausible mechanisms exist: enteroadherence with or without invasion, and enterotoxin or cytotoxin production. We have shown that *A. caviae* is capable of adhering to human epithelial cells without internalization. Consequently, enteroadherence alone, as demonstrated for enteroadherent *Escherichia coli* (15, 20), could serve as the sole virulence mechanism for *A. caviae* as well.

To date, cytotoxin production among aeromonads has been described mainly for *A. sobria* and *A. hydrophila* (3, 19) and has been only occasionally described for *A. caviae* (5). Although it is not the subject of this report, we have shown that cytotoxin production is also a characteristic of *A. caviae* (H. Namdari and E. J. Bottone, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no 904, 1989).

In conclusion, we propose that *A. caviae* (as well as *A. hydrophila* and *A. sobria*) is a bona fide enteric pathogen especially among formula-fed neonates or other subjects whose gastrointestinal tracts provide the requisite milieu

(e.g., pH, redox potential) for survival. We have further shown that *A. caviae* does possess the virulence mechanisms (adherence, cytotoxin production) that may operate in the gastrointestinal tract to provoke a diarrheal syndrome. We additionally stress that the recovery of *A. caviae* from the gastrointestinal tract of any adult with symptoms and a fecal flora which is altered by underlying disease or medication to favor survival raises the potential of *A. caviae* as a pathogen in that setting as well.

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